Food Composition and Acid-Base Balance: Alimentary Alkali Depletion and Acid Load in Herbivores1,2

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Abstract

Alkali-enriched diets are recommended for humans to diminish the net acid load of their usual diet. In contrast, herbivores have to deal with a high dietary alkali impact on acid-base balance. Here we explore the role of nutritional alkali in experimentally induced chronic metabolic acidosis. Data were collected from healthy male adult rabbits kept in metabolism cages to obtain 24-h urine and arterial blood samples. Randomized groups consumed rabbit diets ad libitum, providing sufficient energy but variable alkali load. One subgroup (n = 10) received high-alkali food and 15 mEq/kg ammonium chloride (NH4Cl) with its drinking water for 5 d. Another group (n = 14) was fed low-alkali food for 5 d and given 4 mEq/kg NH4Cl daily for the last 2 d. The wide range of alimentary acid-base load was significantly reflected by renal base excretion, but normal acid-base conditions were maintained in the arterial blood. In rabbits fed a high-alkali diet, the excreted alkaline urine (pHu > 8.0) typically contained a large amount of precipitated carbonate, whereas in rabbits fed a low-alkali diet, both pHu and precipitate decreased considerably. During high-alkali feeding, application of NH4Cl likewise decreased pHu, but arterial pH was still maintained with no indication of metabolic acidosis. During low-alkali feeding, a comparably small amount of added NH4Cl further lowered pHu and was accompanied by a significant systemic metabolic acidosis. We conclude that exhausted renal base-saving function by dietary alkali depletion is a prerequisite for growing susceptibility to NH4Cl-induced chronic metabolic acidosis in the herbivore rabbit. J. Nutr. 138: 431S–434S, 2008.

Introduction

The rabbit is a common laboratory animal for medical basic research in respiratory and renal physiology. However, less attention has been paid in rabbits to the role of food mineral uptake for adaptive functions of acid-base balance. Laboratory rabbit pellet semipurified diet, adapted to the herbivore nutrition habit of this species, is characterized by an alkaline ash: the sum of fixed cations exceeds that of fixed anions, with the difference providing a measure of dietary alkalinity or potential bicarbonate (1,2).

Thus, the rabbit appears to be a suitable animal model for strict herbivore nutrition and may help our understanding of adverse effects caused by extreme base excretion and high urinary pH, e.g., for urinary carbonate stone formation (3). This may be of special interest when alkali-rich diets or therapies are recommended in human medicine to prevent the pathological consequences of nutritional acid load in humans in conditions with impaired renal function, e.g., immaturity in preterm infants (4) or regression in elderly persons (5).

Rabbits normally adapted to alkali-rich nutrition are often investigated for renal responses to chronic metabolic acidosis (6,7), which could only be achieved by ingestion of HCl or NH4Cl when normal feed was withheld, implying concomitant energy deficiency.

The aim of this study is 2-fold: first, to examine the role of high alkali load for urinary pH, bicarbonate excretion, and formation of precipitated carbonates, and second, to investigate the role of strongly reduced dietary alkali load at maintained energy intake for the development of metabolic acidosis induced by ingestion of ammonium chloride with the drinking water.

Materials and Methods

As previously described in detail (2), healthy adult male conscious rabbits (Chinchilla Bastard; Charles River) were investigated, and the experiments were officially approved according to the “Principles of laboratory animal care.” The rabbits were randomly selected for measurements from the central animal care unit of the department, fed an unchanged diet, and individually accustomed to a metabolism cage for at least 1 wk. Daily
food consumption, water intake, and urine excretion were recorded. Data from 122–127 untreated rabbits under control conditions allowed better interpretation of the present results. These reference data were obtained from different series of experiments. They have been partly published elsewhere (2) or are still unpublished.

One subgroup of rabbits (n = 10) received high-energy alkali-rich standard pellets under control conditions, whereby the mean daily energy intake and alkali load were ~395 kJ kg⁻¹ and ~65 mEq kg⁻¹, respectively. During the experimental period of 5 d these rabbits were given a 1% ammonium chloride solution instead of drinking water, corresponding to a mean daily NH₄Cl uptake of 15.4 ± 1.4 mEq kg⁻¹. The other subgroup (n = 14) was fed energy-reduced standard pellets with normal alkali content for control, whereby the mean daily energy intake was ~250 kJ kg⁻¹ and the alkali load ~40 mEq kg⁻¹. To achieve approximately the same energy intake but reduced alkali load during the experimental period, the standard food was changed for 5 d into a commercial high-energy/low-alkali mixture consisting of peanuts, cornflakes, and carob-tree-fruit skin for choice ad libitum. During the last 2 d, this group was given NH₄Cl with water, corresponding to a mean daily NH₄Cl uptake of 4.2 ± 0.4 mEq kg⁻¹. The composition of the 2 standard feed pellets and the low-alkali mixture [Table 1 of Kiwull-Schöne et al. (2)] was determined by analysis of ash in cooperation with the Research Institute of Child Nutrition, Dortmund, and the Institute for Animal Health and Food Quality, Kiel.

**Blood analysis.** Arterial blood samples were taken from the central ear artery under superficial local anesthesia. Arterial pH (pHₐ) and blood gas values were measured by conventional electrodes (ABL 5 Radiometor) at 38°C.

**Urine analysis.** The excreted 24-h urine was collected under paraffin oil to prevent the loss of carbon dioxide. Because the alkaline urine of rabbits eating standard food contained a considerable amount of precipitate, a stirred aliquot was centrifuged for separate analysis of the clear supernatant and the precipitate. The acid-base status of the supernatant was analyzed for carbonate as loss of CO₂ after addition of HCl and back-titratiion with NaOH (2,8).

**Statistical analysis.** Presented data are group means ± SD. After corroborating normal distribution and equality of variances, significant differences between group means were tested by unpaired t tests. The limit of significance was at P < 0.05. Reference values from rabbits not treated with NH₄Cl (see above) underwent regression analysis to obtain relations between selected variables and 95% mean confidence intervals. Statistical analysis was in part carried out using SPSS 8.0 for Windows software.

**Results**

**Effects of different diets on urinary pH and excretion of insoluble compounds.** Rabbits fed species-adapted alkali-rich standard pellets excreted a highly alkaline urine (pHₜ > 8.0), but those fed a low-alkali diet or that spontaneously fasted sharply decreased urinary pH values to the range of 6.0 (Fig. 1). Additionally, within the range of high alkali load, the 95% mean confidence interval of pHₜ for untreated rabbits (n = 127) elucidates urinary acidification both on NH₄Cl ingestion during high-alkali load and on dietary alkali depletion alone. As a result, the alkaline urines contained large amounts of insoluble compounds, up to a daily mean of >1.5 g/kg body weight (2), which became progressively smaller with urinary acidification because of alimentary alkali depletion (Fig. 2), but remained rather unaffected by additional ingestion of NH₄Cl. In comparison, data of an untreated reference group (n = 124) reveal strong reduction of precipitate on urinary acidification because of dietary alkali depletion, but there were no additional effects of NH₄Cl ingestion. According to Flatt and Carpenter (9), rabbit urinary precipitates consist mainly of calcium carbonates (CaCO₃ and CaCO₃·H₂O). On the basis of the average molecular weight of the 2 components, the proportion of carbonates decreased together with the total amount of precipitate, from ~85% on alkali-rich to ~30% on alkali-reduced diets. This is consistent with increasing proportions of phosphate (data not shown).

**Acid-base regulation during alimentary alkali depletion and/or acid load.** In rabbits, a wide range of dietary alkali load can be achieved by varied electrolyte composition of the food and individually different food intake (2). Against this variable nutritional background, acid-base regulation is illustrated for a large number of otherwise untreated rabbits serving as reference groups (Figs. 1–3). The renal response to alimentary alkali depletion comprised urinary acidification and effectively maintained systemic acid-base balance in terms of pHₜ.

![FIGURE 1](image1.png)  
**FIGURE 1** Effect of alimentary acid-base load on renal acidification in rabbits. Ordinate: Urinary pH (pHₜ) values. Abscissa: Dietary acid-base load calculated from the feed’s ash cation-anion difference and daily food intake ad libitum (2). Mean values ± SD of the subgroups ingesting ammonium chloride (NH₄Cl) either with high-alkali food (n = 10) or with low-alkali food (n = 14) differ significantly (P ≤ 0.01).

![FIGURE 2](image2.png)  
**FIGURE 2** Effect of renal acidification on excretion of insoluble compounds in rabbit urine. Ordinate: Daily excreted amount of precipitate. Abscissa: Urinary pH (pHₜ) values. Mean values ± SD of the subgroups ingesting ammonium chloride (NH₄Cl) either with high-alkali food (n = 10) or with low-alkali food (n = 14) differ significantly (P ≤ 0.01).
Values. Abscissa: Dietary alkali load defined as in Figure 1. Mean 
SD of the subgroups ingesting ammonium chloride (NH4Cl) 
either with high-alkali food (n = 10) or with low-alkali food (n = 14) 
differ significantly (P < 0.01).

As long as the high-alkali nutritional background prevailed, 
a high cumulative NH4Cl load by as much as 75 mEq kg−1 over 
5 d significantly decreased pHa to 7.00 ± 0.32 (n = 10, P < 
0.001) compared with 8.09 ± 0.30 (n = 58) in the reference 
group (2) but did not impair the arterial pH values (Fig. 3). On 
low alkali intake, however, a much smaller cumulative NH4Cl 
challenge of only ~0.1 (8 mEq kg−1 for 2 d) did cause a sig-
nificant acidosis in the arterial blood (P < 0.01), decreasing pHa 
to 7.342 ± 0.052 (n = 14) compared with 7.418 ± 0.031 (n = 
19) on alkali reduction alone. Under the latter condition with 
and without NH4Cl load, urinary pH values did significantly 
(P < 0.01) but not greatly differ between 5.69 ± 0.45 and 
6.32 ± 0.58, respectively. Furthermore, the 95% mean confi-
dence interval of pHa for a large range of dietary alkali load in 
untreated rabbits (n = 122) (Fig. 3) illustrates that additional 
NH4Cl ingestion does not impair arterial pH homeostasis as 
long as high alkali supply is provided, but considerable systemic 
acidosis develops as a result of (even much smaller amounts of) 
ingested NH4Cl on concomitant dietary alkali depletion.

Thus, in the rabbit it is only possible to elicit a manifest 
NH4Cl acidosis against the background of low-alkali nutrition.

**Discussion**

The impact of herbivore alkali-rich nutrition on kidney function. Rabbits on species-specific alkali-rich standard semi-
purified diet excrete highly alkaline urines but develop significant 
urinary acidification on dietary alkali depletion. Consequently, 
alkaline urines of rabbits contain large amounts of insoluble 
compounds, mainly consisting of calcium carbonates (9), which 
progressively disappear at lower values of pHa. The precipitated 
carbonate must be considered a portion of total base excretion 
(2), e.g., for quantitative estimation of urinary net acid excretion 
during NH4Cl challenges.

Interestingly, large amounts of calcium carbonate crystals are 
not harmful to the urinary tract of the rabbit because they form a 
soft chalk-like matter and are covered with mucus that is excreted 
by specialized glands (10). In contrast to these adaptations in the 
herbivore rabbit, vegetarian nutrition in omnivore species such as 
humans may enhance the risk of calcium carbonate stone for-
mation, at least under extreme conditions such as progredient 
bone demineralization, e.g., from immobilization during pro-
longed bed rest or weightlessness in space labs (11).

The role of nutritional alkali background for the manifes-
tation of an experimental ammonium chloride acidosis. In the 
herbivore rabbit, systemic acid-base balance is normally 
maintained over a wide range of nutritional alkali load by ap-
propriate renal base reabsorption (2). The present results have 
shown that it is not possible to induce a systemic metabolic 
acidosis in rabbits even by high-dose application of ammonium 
chloride under normal herbivore nutrition. The resulting distinct 
renal acidification, which likely is a base-saving response, 
appears rather unexpected in view of persisting high alkali intake 
but may explain systemic acid-base homeostasis. However, prior 
stress on renal acid-base control by alimentary alkali depletion, 
leading to nearly complete base reabsorption (2), elicits growing 
susceptibility to chronic metabolic acidosis in the rabbit. In 
agreement with observations on preterm infants (12), and based 
on supplementary yet unpublished observations, our data predict 
significant reduction of arterial pH (and base excess) for net acid 
excretion values above 0 at a pHa level below ~6.5.

Because of the diet’s high energy content, our approach has the 
further advantage of sufficient energy supply in addition to alkali 
depletion, so that adverse effects on respiration and metabolism, 
also involved in acid-base balance, can be avoided. Up to now, 
investigators attempting hyperchloremic metabolic acidosis in 
rabbits performed concomitant food deprivation (6,7).

There is general agreement that HCO3− reabsorption by proximal tubules is the leading process in renal acid-base regu-
lation (13). At the molecular level, many functions of isolated 
kidney tubules have been studied in the rabbit (7,14), which has 
shown that carbonic anhydrase II and IV in different nephron 
segments are stimulated during chronic metabolic acidosis. Like-
wise, the sodium-proton exchanger subtype NHE3 is prof-
doundly involved in proximal tubular bicarbonate retention and 
abundance and activity of the NHE3 protein are also regulated, 
lowered by alkali load (15) and enhanced by NH4Cl-induced 
acidosis (16,17). Recently, we have shown that chronic meta-
bolic acidosis in rabbits did cause NHE3 mRNA up-regulation 
not only in the kidney but also in brainstem regions involved in 
central respiratory control (18).

The interplay of ion-exchange proteins and enzymes for acid-
base homeostasis in rabbits exposed to NH4Cl against the 
background of high and low nutritional alkali load remains to be 
clarified.

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